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Instructions for use

A Karyological Investigation of *Mitrastemon* Yamamotoi Mak., with Special Reference to the So-called 'Diffuse Stage' in Meiosis'

 $\mathbf{B}\mathbf{y}$

HAIIME MATSUURA

(With Plates V-VIII and 18 Text-figures)

Since Makino's description in 1909 and subsequent establishment of a new taxonomic order by him ('11), and because of the peculiarities in its morphological characteristics and habit of growth, *Mitrastemon Yamamotoi* Mak, has received much attention from botanists.

The writer has been carrying on an investigation involving the cytology and anatomy of this plant, with an aim to render some contribution to our knowledge on its mode of development. A preliminary note was read on the pollen development in this plant at the Annual Congress of the Botanical Society of Japan held in the spring of 1932. A demonstration was given there on the so-called 'diffuge stage' in meiosis of the PMC.

The present paper is the result of the further and completed study on the same subject, together with some additional data from observations on meiosis in the EMC.

1. Material and Methods

The material studied was collected by the writer in two successive years, October 1931 and November 1932, from Yunomoto near Kagosima, where the plant is abundantly found flourishing on the roots of a species of Shiia (S. Sieboldii?).²⁾ The appearance of the plant as it grows there is shown in Text-fig. 1. The fixation and subsequent treatment of the material were carried out at the same point while the writer was staying there. The meiotic division of the PMC was found to take place early in October when the anther was closely enclosed by the perianth.

¹⁾ Aided by a grant from the "Nippon-Gakuzyutu-Sinkokwai" (the Foundation for the Promotion of Scientific and Industrial Research of Japan).

²⁾ Some material was also collected from the same place and sent to the writer by Prof. Kimura of the Tôhoku Imperial University, to whom the writer wishes to express his hearty thanks.

The fixing fluids used were weak Flemming's, Carnoy's and Navashin's fixatives, of which the last usually proved to give the best results. Sections were cut at a thickness of about 10 μ and most of the preparations stained with gentian violet after Newton's method. The writer experienced much difficulty in obtaining satisfactory results for staining the nucleus of early meiotic stages. After several treatments were tried, it was found that the preparation immersed in 1% chromic acid solution at 50°C for several hours, followed by staining with gentian violet gives good results. Pl. VI, Figs. 3–7 were taken from preparations of such treatment.



Text-fig. 1. Mitrastemon Yamamotoi growing on the roots of Shiia in Yunomoto, Kagosima Prefecture. Photo, by Kimura, Nov. 2, 1931.

2. The Chromosome Number

The number of chromosomes in *Mitrastemon Yamamotoi* Mak. was found to be $n=20^{10}$ and 2n=40. The diploid number was counted at the division of nucellus cells (Text-fig. 2). The haploid number was determined in metaphase of meiosis in both PMC and EMC (Text-figs. 3 and 6). The number was also counted in the first vegetative division of a megaspore. Clearly 20 chromosomes were distinguished there in both daughter nuclei (Text-fig. 5). Some variation in the bivalent number, however, has been observed in both the PMC and EMC. This will be discussed later.

¹⁾ The haploid number was recently ascertained by Watanabe ('34). No figure however has been given as yet.



Text-figs. 2-6. 2, somatic chromosomes. 3, polar view of metaphase I plate in a PMC. 4, polar view of anaphase I in a PMC. 5, first division of a megaspore, taken from two successive secctions. 6, side-view analysis of the metaphase I chromosomes in an EMC. 2-5, ×1400; 6, ×2800.

It may be noted *en passant* that in *Rafflesia Patma*, a species to be considered as an allied one, the haploid chromosome is 12 (ERNST & SCHMIDT, '13).

3. Meiosis in the PMC

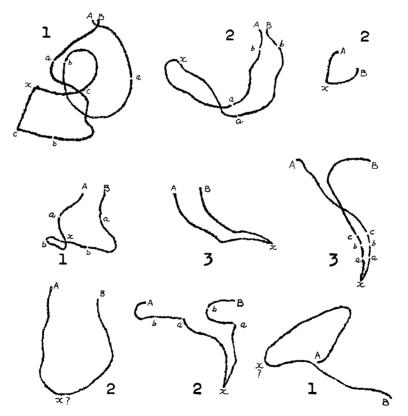
Prophase. Some characteristic features were noted in the prophase of the PMC., (1) the absence of synizesis, (2) the chromosome pairing with complete polarization and (3) the occurrence of the so-called 'diffuse' stage.

The absence of synizesis in the PMC makes a contrast to the prophase of the EMC, where the synizetic stage is regularly found. This may be due to differences in effects of fixation, the EMC being naturally subjected to more prolonged infiltration of fixative fluids owing to the larger amounts of surrounding tissues. It must be noted however that no literature has been published, so far as the writer is aware, demonstrating such a difference in the PMC from that of the EMC, and moreover that, as suggested from the previous paper on *Phacellanthus* (Matsuura, '35), the stage at question is at least naturally characterized by shrinkage of the nuclear content and subsequent migration of the nucleus. It will be then inferred with safety that in the present material the PMC differs from the EMC in showing a less tendency of shrinkage at this stage.

At the onset of meiosis in the PMC, the dark staining irregular chromatic bodies in the resting stage resolve themselves into threads of vague contour which very soon (perhaps) become more even and distinctly outlined (Pl. VI, Figs. 3 and 4). The leptotene thread thus formed is fairly thick, having a decidedly granular appearance. It consists of separate segments, not continuous as known in many plants. Unfortunately the leptotene stage presented considerable difficulty in its analytic study, partly owing to the rapidity with which the pachynema formation takes place and partly owing to the presence of some long entangled threads which were difficult to trace. The writer, however, could demonstrate that end-to-end conjugation of threads two-by-two forms V-shaped Text-fig. 7 represents nine threads at zygotene which were taken from three different nuclei. The point of union of the threads is marked "x", the free ends "A" and "B". Some of the threads are characterized by constricted parts which were marked "a", "b", "c", etc. In these cases, the respective positions of the constrictions were found to be entirely correspondent with each other in each of the arms of the V. Though it was impossible to identify all the spiremes in a complement, it seemed

very probable that they are present in not more than the haploid number, viz. 20. Consequently the two arms of each loop are considered to constitute a pair of homologous chromosomes lying side by side. The pairing of threads seems to take place very rapidly, since no Y-shaped figures were found with longitudinal double stems, from which diverge the two halves to form the single arms of the Y. This led the writer to assume the telosynaptic mode of chromosome pairing, but owing to considerable difficulty in the complete study of these stages, a final conclusion must be postponed.

The pachytene threads thus formed are characterized by distinct polarization with their ends towards one pole of the nucleus where the nucleolus is usually present (Pl. VI, Fig. 6). This is regarded as the pachytene bouquet stage. The spiremes contract, becoming very short and

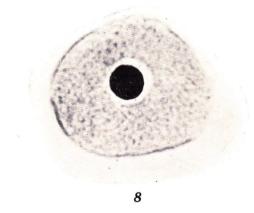


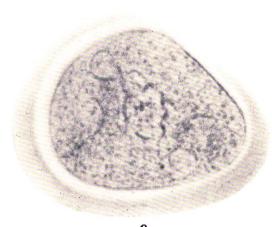
Text-fig. 7 Representing 9 spiremes at zygotene, taken from 3 different nuclei, 1, 2 and 3. $\times 4000$.

they are distributed throughout the nuclear area. Pl. VI, Fig. 8 represents a stage which may be taken as the diplotene.

Very characteristic is a stage directly following the diplotene stage just described. It corresponds entirely to the so-called 'diffuse' stage, a condition so generally found in meiosis in animals but not hitherto described in higher plants. Features of this stage may be described in the order of events as follows:

i) At the onset of 'diffusing', the spiremes show a more or less diminished basophily and become so elongated that they acquire more vague contours (Pl. VI, Fig. 9).





Text-figs. 8 and 9. 8, a 'nucleusless' PMC. 9, a PMC in which the nucleus was completely decomposed. × 2000.

- ii) As the loosening up of the spireme-threads proceeds, they take a marked fine beaded structure and become distributed evenly in the nuclear area (Pl. VI, Fig. 10).
- iii) The affinity for dyes is more and more diminished, leaving only a few small chromatin granules visible here and there (Pl. VI, Figs. 11 and 12).
- iv) Finally the spiremes are lost to view in a general nuclear network (Pl. VII, Fig. 13).

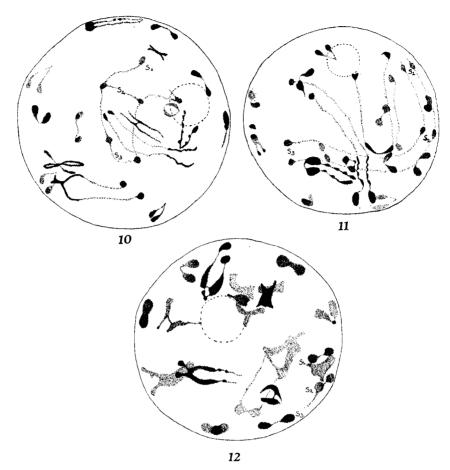
At this final stage, the diffusing process attains its maximum, and the plasms outside and inside the nucleus become identical in appearance, being delimited only by a very delicate nuclear membrane. That the condition of the plasm in the nucleus approaches very closely to that of the cytoplasm at this stage is shown

by the frequent occurrence of mother-cells where the nuclear mebrane is entirely lost and only a dark-staining chromatin mass surrounded by homogenous plasm takes the position of the nucleus (Text-fig. 8).

Such 'nucleus-less' mother-cells are obviously functionless and remain for a somewhat long time as such. Later on, however, in some of them the chromatic mass appears to be decomposed and absorbed by the surrounding plasm which becomes vacuolated to a high degree (Text-fig. 9).

Diakinesis and Metaphase. Generally speaking, the order to reappearance of the chromosomes is the reverse of that in which they disappeared at the diffuse stage. The first indication of chromosome reformation is the appearance of very small chromatic granules which are sharply defined in outline and lie usually side by side (Pl. VII, Fig. 14). They are few at first, but gradually increase in number and at the same time in size (Pl. VII, Fig. 15). At a certain stage, such as represented by Text-fig. 10, most of the bivalents are seen to be definitely formed, but some of them are still in the process of concentration. In some of the chromosomes the concentration takes place at several points, which sometimes are situated very far from each other, thus separating the entire chromosome into some chromatic masses which remain connected by fine strands. As the process of concentration proceeds, these chromatic masses come together and form well defined bivalent chromosomes.

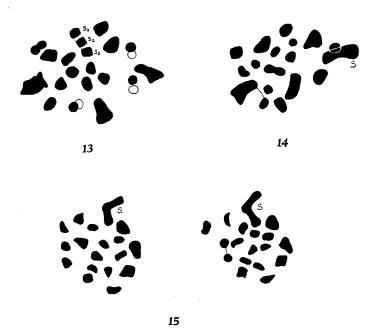
Another peculiarity noted in the diakinetic nuclei is the occurrence of three small bivalents connected with one another by fine strands, as represented by s₁, s₂ and s₃ in these figures. Taking the condition as similar with large bivalents which are characterized by more than one point of concentration, they may be considered to represent one pair of large chromosomes in which the concentration takes place at three different points located far from one another. This would lead one to predict the formation of 18 bivalents at metaphase. Contrary to this expectation, most of the metaphasic nuclei examined revealed 20 bivalents, as already Frequently, however, a marked tendency of association of three chromosomes was noticed (Text-fig. 13). The figure suggests the secondary association of chromosomes predicted by LAWRENCE ('31), but the present case appears to be of a quite different origin. Moreover only one amongst about 20 nuclei examined showed 18 bivalents including a very large one (Text-fig. 14), suggesting that the three chromosomes were fused together. Such numerical variation of chromosomes was also observed in nuclei at anaphase; most cases showed 20 chromosomes in each of the daughter nuclei (Text-fig. 4); one case, 19 chromosomes in one and 20 in the other nucleus;



Text-fig. 10-12. Three diakinetic nuclei, showing three successive stages of chromosome reformation after 'diffusing'. $\times 4000$.

one case, 18 chromosomes in each (Text-fig. 15). The last case clearly indicated that the largest chromosome was composed of three elementary ones.

The variation in chromosome number in the present case is especially interesting in connection with the findings by Youngman ('31) on the numerical relationship between the spiremes and the chromosomes in the PMC of *Thespesia*. He found that there are only 8 spireme loops in early phases of meiosis, and from them 13 chromosomes result at metaphase I. It is then inferred that 10 of these 13 "have been derived from the five longer loops by transverse fission of each into two," and "the other three



Text-fig. 13-15. 13, polar view of metaphase I in a PMC; the three small bivalents (s_1 , s_2 and s_3) are connected by fine strands. 14, polar view of metaphase in a PMC; a large bivalent (marked S) is considered as having originated from the fusion of three small bivalents. 15, two anaphasic plates in a PMC; the largest chromosome in each plate (S) has resulted from the fusion of three small ones. $\times 1400$.

have come one from each of the other three loops". In the present material, the number of spiremes in the early stage of meiosis was not determinable, but in analogy with *Thespesia*, it would be possible to assume that the spiremes in *Mitrastemon* may be 18 in number, one of which gives 3 chromosomes at metaphase when the transverse fission is complete, 2 'chromosomes' when the fission is incomplete, and 1 'chromosome' when no fission takes place.

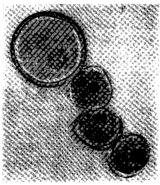
Interkinesis and onwards. As the arrival of half dyads at each pole of the heterotypic spindle is completed, they become closely grouped together and lose their sharp outline (Pl. VII, Fig. 17). Pl. VII, Fig. 18 represents a later stage in which each daughter nucleus forms a surrounding membrane and the formation of a loose and heavy threadwork is taking place within it. This process is much advanced and on the threadwork develop a number of small chromatin globules, one of which is clearly larger than others and probably represents a nucleolus. The aggregation

of chromatin proceeds further and at the same time the number of aggregates is considerably diminished. The ground threadwork becomes distinctly oxyphilic. Such a condition is represented in Pl. VII, Fig. 19, in which only a small number of chromatin aggregates and one nucleolus are formed in each daughter nucleus. Thus the reformation of the nucleolus is in a manner quite similar with the other chromatin aggregates, being distinguished only from the latter by usually having a crystalline body within it. The number of chromatin globules appeared to be constant at the maximum condition of this stage when usually five large globules were counted. A striking correspondence was noted as to their size and sometimes their position in each daughter nucleus.

Then the chromatin aggregates including the nucleolus appear to undergo a sudden change; they become completely lost to view in the nuclear network and the whole structure becomes entirely oxyphilic. In the extreme condition, only a nucleolar inclusion and a vague contour of nucleolus surrounding it is visible in each nucleus, the other structure being entirely non-traceable. This condition may be taken as the second diffusion; however the degree of it is more intense than that of the first diffusion where the nucleolus retains its basophyly throughout.

The reformation of the chromosomes is accomplished in a similar way as that in which the bivalent chromosomes are formed after the first diffuse stage. They appear first as small tadpole-shaped bodies within the nucleus which gradually grow up and finally take on a sharp outline (Pl. VII, Fig. 20). During these stages the nucleolus and its inclusion disappear completely.

During metaphase and early telophase the chromosomes become very



Text-fig. 16. Three normal pollen-grains and one giant grain. Aceto-carmine. × 500.

compact and closely associated, making the identification of individual chromosomes impossible. This is probably due to temporary coalescence of chromosomes and not to true fusion.

At the reconstruction of the grand-daughter nuclei, the chromosomes become elongated considerably and eventually appear to lose their outline in the nuclear area around which a delicate membrane is formed. The tetrad formation proceeds in the ordinary simultaneous way. The mature pollen is binucleate.

Text-fig. 16 represents four pollen-grains, one of which is considerably larger than the others and chracterized by a degenerated nucleus. Such a giant pollen may have originated from the disappearence of the nuclear membrane at the diffuse stage and may be functionless.

4. Supplementary Observations on Meiosis in the EMC

In comparison with the meiotic processes in the PMC, some detailed studies have been done of those in the EMC, with special reference to the diffuse stage.

The early development of the EMC is characterized by the occurrence of synizesis (v. p. 192). At the initiation of this stage, the fine leptotene threads begin to migrate in the nuclear cavity toward one side of the nucleus, and later on form a densely staining knot (Pl. VIII, Fig. 25). Owing to the occurrence of this stage where the synizetic knot is so tight that no individual threads could be identified, the present case is more unfavorable in deciding the exact mode of thread pairing than that in the PMC. It may be also noted that the synizetic stage of the EMC appears to be of somewhat long duration as judged from a considerable number of preparations showing this stage.

Another characteristic feature in the prophasic phases of the EMC is the distinctly earlier initiation of the diffuse stage as contrasted with that of the PMC. As the thickened threads emerge further throughout toward the periphery of the nuclear cavity, they gradually lose their outline and become distinctly oxyphyly (Pl. VIII, Fig. 28). The later processes of 'diffusing' are quite correspondent to those in the PMC as seen in Pl. VIII, Figs. 29 and 30.

The reformation of the chromosomes was found to take place in a similar way with that observed in the PMC; they appear themselves first as minute chromatic bodies near the periphery of the nucleus (Pl. VIII, Fig. 31) and as the recondensation proceeds further, they take a distinct outline as bivalents (Pl. VIII, Fig. 32).

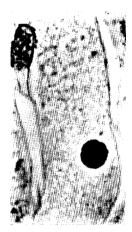
At metaphase, 20 bivalents can be generally distinguished. Text-fig. 6 represents a complete side-view of a metaphasic figure. Owing to the greater area of the nuclear plate in the EMC, it is easier to analyze the chromosome configuration than in the PMC. At anaphase 20 half-dyads were also generally observed; in one case, however, only 18 chromosomes were counted in both the daughter nuclei, and in still another case, one of the daughter nuclei possessed only 18, the other, 20 chromosomes (Text-fig. 17). These variations in chromosome number are considered to be

due to the temporary fusion of the three elementary chromosomes, as described in the PMC.



Text-fig. 17. Anaphase I in an EMC. In the right plate, a large chromosome (S) is supposed to have originated through fusion of three small chromosomes. ×1400.

Pl. VIII, Fig. 33 represents a phase of interkinesis. Similarly to the case in the PMC, a large nucleolus containing an inclusion, together with several chromatic aggregates, can be distinguished. One of the daughter cells which is



Text-fig. 18.
A 'nucleus-less' EMC.
× 1100.

situated near the microphylar end appears to degenerate rapidly, thus division II takes place at the lower nucleus only. The result is the formation of a row of three megaspores. The outer one degenerates, while the innermost cell grows further and develops the female gametophyte.

Text-fig. 18 represents a 'nucleus-less' EMC which appears to have originated at the diffuse stage, a condition entirely similar to that already described in the PMC.

5. On the Diffuse Stage

As may be seen from the above description, the most striking feature in the meiocyte of *Mitrastemon* is the regular occurrence of the so-called diffuse stage just prior to diakinesis. At this stage the diplotene threads are entirely lost to sight and the general appearance of the nucleus approaches closely to that at the resting stage. In a few lower plants, similar peculiarities have been described in their meiocytes, *i.e.*, *Dictyota* (WILLIAMS, '04), *Padina* (CARTER, '27) and *Equisetum* (LENOIR, '32), but no such extreme case of 'diffusing' has been demonstrated in higher plants, although a similar but less-noticeable modification of the threads is sometimes said to occur at the corresponding stage.

Wilson ('28, p. 350) distinguishes several grades of diffusing in animal

meiocytes. The present case corresponds to his 'third class' of diffusing which is described as follows: "A third class includes those still more extreme cases in which the chromosomes are finally completely lost to view in the nuclear network (dictyotic stage of Winiwarter) and the whole structure becomes nearly or quite oxyphylic. The classical case of this is offered by the amphibia,". The present writer however is not aware of any case in which the diffusion proceeds so far, as in the present material, that at this stage the frequent occurrence of disappearance of the nuclear membrane follows, thus giving rise to 'nucleus-less' cells.

It is the general conviction that the occurrence and grades of 'diffusing' are distinctly correlated with the increase of cytoplasmic volume; for 'in general the longer the growth-period and the greater the growth of the auxocytes the greater the nuclear diffusion' (Wilson, id. p. 545). In the animal occyte, the growth-period is generally more extensive and consequently the diffusing process is more pronounced, than in the spermatocyte. In these connections, the following findings in the present material will be of special interest:

- 1) As already described, the diffusing process in the EMC does not differ in its grade from that in the PMC.
- 2) The comparison of the size of PMC at pre- and post-diffuse stages with that at the diffuse stage (Table I) clearly indicates no apparent increase in cytoplasmic volume during the stage at question. The figures in the following table represent average areas of the largest section of cells and nuclei, measured by the aid of a planimeter. The apparent gradual decrease in the area of cells towards the diffuse stage seems most probably to indicate that the cell is changing its form from a two dimensional to a three dimensional one. It is also worthy of notice that the size of the nucleus is distinctly diminishing towards the diffuse stage.

TABLE I
Comparison of the size of PMC and nuclei in *Mitrastemon Yamamotoi* MAK.
(Measurements in square mm. when magnified ×1250 are shown in brackets)

Stages	Cells	Nuclei
Resting	486.4 ^{□µ} (76)	$160.0^{\Box \mu} (25)$
Pachytene	467.2 (73)	166.4 (26)
$\mathbf{Diffuse}$	428.8 (67)	134.4 (21)
Early diakinetic	480.0 (75)	140.8 (22)

Each figure given is the average of 20 measurements.

These findings lead one naturally to the view that at least in the present material the nuclear diffusion is not so correlated with the cytoplasmic growth, as hitherto believed in animal auxocytes.

A question may be raised next: whether or not the nuclear diffusion is correlated with the parasitic nature of the plant? The comparison of several other plants on this point seems to reveal its diverse nature. an allied species, Rafflesia Patma, Ernst & Schmidt ('13) have given no description on the nuclear diffusion in its meiocyte. In Phacellanthus tubiflorus, a member or Orobancaceae, the writer also found no occurrence of such a stage. It is interesting however to note here that in meiosis of PMC in Lathraea, another member of Orobancaceae, Gates & Latter ('27) describe a peculiar stage just prior to diakinesis in which "the large chromatic masses appear suspended in the nuclear cavity by the delicate strands of reticulum". An inspection of their Pl. III, Figs. 21 and 22 seems to point to another kind of diffusion occurring at this stage, suggesting Wilson's 'fourth group' of diffusion which is characterized by the karyosphere-formation, although the original authors take this stage as "an intermediate position between typical parasynapsis and telosynapsis" and consequently as "a lowly type of nuclear specialisation which may perhaps be correlated with the parasitic nature of the plant". In this connection, it must be added that the present writer has found in the meiocyte of Balanophora tobiracola Mak. (unpublished), a condition quite similar with Lathraea, a close observation on which seems to favor the writer's interpretation that it may represent another kind of nuclear diffusion.

6. Summary

- 1) In the present study, *Mitrastemon Yamamotoi* Mak. has been treated karyologically during both micro- and macrosporogenese.
 - 2) The chromosome number was determined as 20 haploid, 40 diploid.
- 3) At metaphase I, three small bivalents tend to fuse together, thus resulting in apparent variation of the bivalent number from 18 to 20.
- 4) The most characteristic feature in the prophasic phases of both PMC and EMC is the occurrence of the nuclear diffusion. It occurs after the diplotene stage and prior to diakinesis. At this stage, the spiremes elongate considerably, take vague contours and become entirely oxyphylic, a condition approaching closely to the nucleus at the resting stage.
- 5) No correlation has been found between the occurrence of the nuclear diffusion and the increase of cytoplasmic volume.
 - 6) At the diffuse stage, the nuclear membrane may be sometimes

entirely lost, giving rise to the formation of functionless giant pollengrains. The same phenomenon takes place in the EMC.

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Explanation of Plates V-VIII

All the photos were taken with the aid of a Leitz microphotographic apparatus MAII under 5 amp. D.C. are light, and have been reproduced without reduction. Figures in Pl. V were taken under a Leitz 4 with Zeiss comp. oc. $10 \times (\text{magnification } ca\ 210)$; figures in Pl. VI and VII were taken under a Zeiss apoch. obj. $120 \times (\text{N.A. } 1.3)$ with Homal IV (magnification $ca\ 2000$) and those in Pl. VIII under the same oc. with Leitz 1/12 oil imm. obj. (magnification $ca\ 1600$).

Abbreviations

C: Carnoy's fixative.

Fw: Flemming's weak fixative.

N: Navashin's fixative.

Plate V

- Fig. 1. A part of a young anther in face view, containing PMC in its loculi at the diplotene stage. (C)
- Fig. 2. Cross-section of a part of an anther, containing PMC at the diffuse stage. (N)

Plates VI-VII

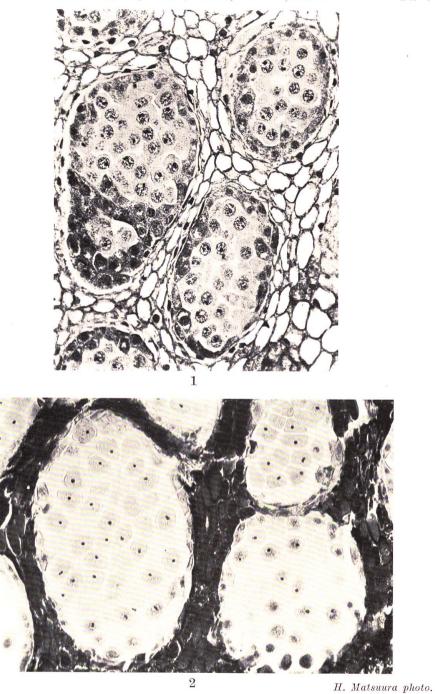
Meiosis in the PMC.

- Fig. 3. A resting PMC with chromatic aggregates held in the meshes of the fine reticulum in the nucleus. (C)
- Fig. 4. A nucleus at the initiation of the prophase, the chromatin aggregates resolving themselves into slender threads. (C)
- Fig. 5. A nucleus at a somewhat later stage. (C)
- Fig. 6. A nucleus at the pachytene stage. (C)
- Fig. 7. Thickening of the spiremes. (Fw)
- Fig. 8. A nucleus at the diplotene stage. (Fw)
- Fig. 9. Initiation of disorganization of the spiremes. (Fw)
- Fig. 10. Uniform distribution of the threads throughout the nuclear cavity. (N)
- Fig. 11. A nucleus at early diffuse stage. (N)
- Fig. 12. Later stage, with only a few chromatin granules faintly stained. (N)
- Fig. 13. Typical diffuse stage. (N)
- Fig. 14. Recovering from the diffuse stage, and chromosome recondensation. (N)
- Fig. 15. A nucleus at early diakinesis. (N)
- Fig. 16. Mid-metaphase; same as Text-fig. 13. (N)
- Fig. 17. A PMC at anaphase I. (N)
- Fig. 18. Initiation of interkinesis and formation of nuclear membrane in each daughter nucleus. (N)
- Fig. 19. Interkinesis. (N)
- Figs. 20 and 21. Reorganization of chromosomes in one of the daughter nuclei. (N)
- Fig. 22. Formation of pollen tetrad nuclei. (N)
- Fig. 23. A young microspore. Formation of resting reticulum of the nucleus.

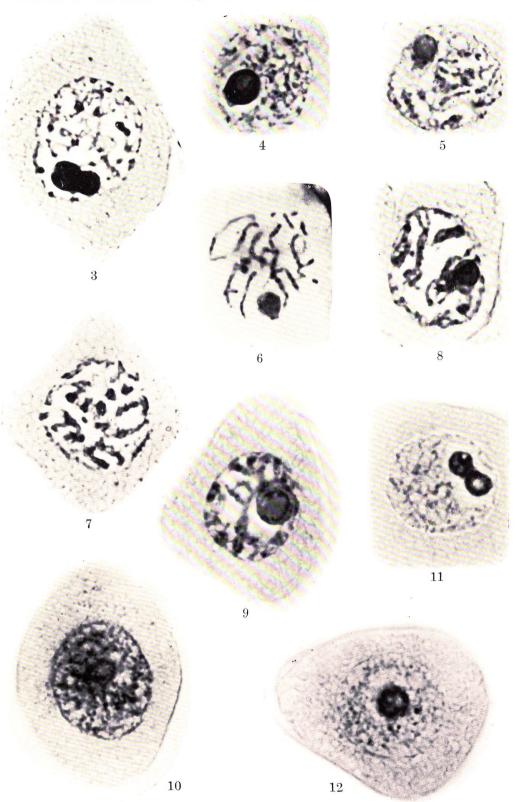
Plate VIII

Meiosis in the EMC.

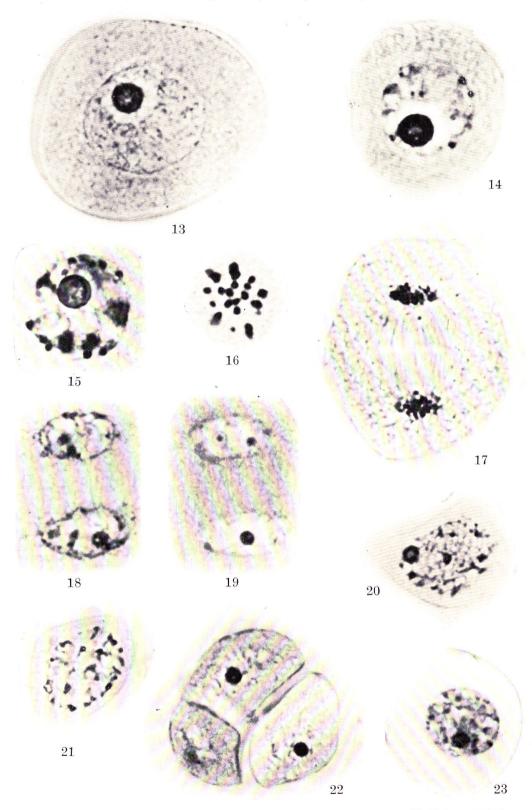
- Fig. 24. Initiation of prophase. (N)
- Fig. 25. Early synizetic stage. (N)
- Fig. 26. Late synizetic stage. (N)
- Fig. 27. Formation of thick spiremes. (N)
- Fig. 28. Initiation of the diffuse stage. (N)
- Figs. 29 and 30. Diffuse stage. (N)
- Fig. 31. Appearance of the centers of chromosome recondensation. (N)
- Fig. 32. Early diakinesis. (N)
- Fig. 33. One of the dyad nuclei, showing a nucleolus containing a large inclusion, and several chromatic aggregates. (N)
- Fig. 34. Division II. (N)
- Fig. 35. First vegetative division of a megaspore, showing 20 haploid chromosomes; same as the right figure in Text-fig. 5. (N)



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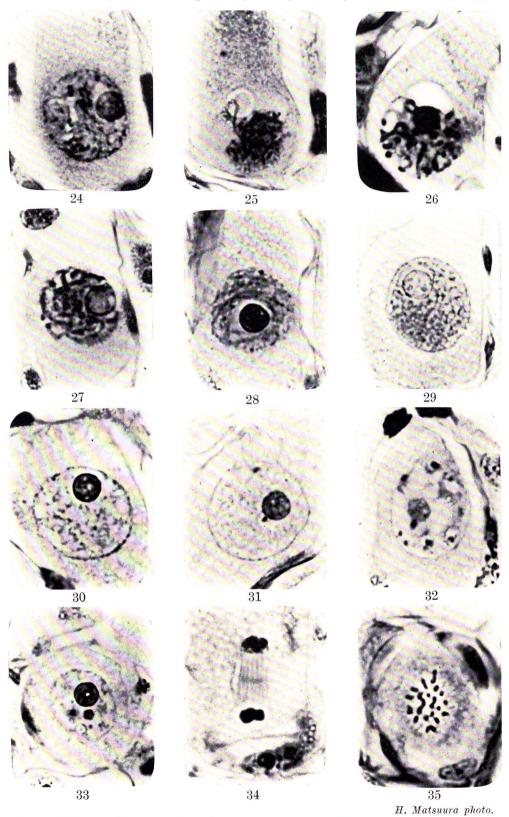


 $H.\ Matsuura\ photo.$ H. Matsuura photo. H. Matsuura: A Karyological Investigation of $Mitrastemon\ Yamamotoi\ Mak.$



H. Matsuura photo.

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